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Comparative molecular and physiological analyses of low salinity adaptation between closely related *Ulva* species in marine and brackish water.

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Organisms interact with their environment such as temperature, light intensity, and salinity. For aquatic organisms, the salinity concentration is the key factor among various environmental factors. Ionic concentrations and osmotic pressure of marine water and those of fresh water are different. Therefore, invasion of organisms from marine water to fresh water has led to rapid morphological and physiological adaptations. The low salinity adaptation is interesting about molecular mechanism and molecular evolution. However, studies on the tolerance to low-salinity condition have not been conducted, because there are few organisms which are most suitable for this study.

Ulva prolifera is commonly found in estuaries and brackish waters, whereas *Ulva linza* is found only in seashore habitats. Phylogenetic analysis of ITS2 nrDNA region sequences have shown that *U. prolifera* and *U. linza* are closely related species. And male gametes of *U. linza* could successfully fertilize female gametes of *U. prolifera*. Moreover, hybrid zygotes can normally develop into sporophytes with meiotic capability. Despite this degree of relatedness, *U. prolifera* and *U. linza* exhibited a physiological difference in fresh water environment. It was reported that the cell viability of *U. linza* in fresh water dropped to approximately 20 % after 7 days, while that of *U. prolifera* remained as close as 100 %. This difference in tolerance to low salinity makes the genetically closely related *U. prolifera* and *U. linza* a good pair of model species in which to study adaptation to low salinity.

To understand low salinity adaptive evolution that contributed to increasing species diversity, the purpose of this study is to understand the low-salinity adaptation of *U. prolifera* from the point of molecular and physiological view. In chapter 1, the comparative RNA-seq analysis was conducted. In chapter 2, we measured the growth rate and analyzed the intracellular chemical elements using ICP-MS.

In chapter 1, we sought to identify genes involved in low-salinity adaptation in *U. prolifera* by comparison of the gene expression profiles of *U. prolifera* and *U. linza* cultured under marine water, brackish water and fresh water conditions. When both *U. linza* and *U. prolifera* were transferred to fresh water condition from sea water condition, they upregulated DASS transporter genes whose function was transporting sulfur. *U. linza* further upregulated genes for heat shock

proteins for survival but the organisms were unable to survive in fresh water. In contrast, *U. prolifera* further upregulated genes for the cell wall hydrolases to adjust cell turgor and downregulated the genes for lipid metabolic processes.

In chapter 2, to measure the growth rate of both *Ulva* species, thalli were cultured under 0, 5, 10, 15, 20, 25 and 30‰ salinity conditions. The growth rate at 0‰ salinity condition was the highest in *U. prolifera*. But no differences between the growth rates were statistically significant. In *U. linza*, the growth rate at 15‰ was the highest and that at 0‰ was the lowest. The differences of the growth rates were statistically significant against the ones in other conditions. To measure the chemical element content, thalli were cultured under 0, 5, 15 and 30‰ salinity conditions. Boron, sodium, magnesium, phosphorus, sulfur, potassium, calcium, manganese, iron, copper and zinc were analyzed. We found that the total chemical element contents of *U. linza* under 5, 15 and 30‰ were higher than that of *U. prolifera* under 30‰ by intra-specific comparison of the total chemical element contents. In *U. linza*, total element contents under 5, 15 and 30‰ salinity conditions did not show significant difference, and in *U. proilfera*, the total chemical element contents gradually decreased as the salinity condition outside becomes low. These results suggest that the amount of the chemical elements necessary for survival in *U. linza* should be much higher than that in *U. prolifera* and that the amount should be kept in constant quantity regardless of the external salinity conditions.

Unifying the results of chapters 1 and 2, we could hypothesize that low salinity adaptation in *U. prolifera* might be realized by succeeding in making up for sulfur which was short under low salinity condition by upregulating DASS transporter, reducing strength of the cell wall by upregulating cell wall hydrolase, and downregulating genes for lipid metabolic process under low salinity condition. As a result of these gene expressions, *U. prolifera* can grow without being affected by the change of external salinity conditions from fresh water to seawater, and can survive in low salinity environment like estuary for a long period, and then, *U. prolifera* might have evolved from *U. linza,* the marine species. For future study, to confirm the expression state and the functions of genes selected in this study, microarrays, RT-PCR and the gene recombination studies should be performed. The mechanisms of the adjustment of its chemical element contents are also in need to be elucidated for full understanding on the low salinity adaptation of *U. prolifera*. This new knowledge will contribute to the detailed understanding of speciation brought by low salinity adaptation evolution in plant lineages.